The rise and fall of Sauropus (Phyllanthaceae) : a molecular phylogenetic analysis of Sauropus and allies

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Chapter 1

General Introduction

This thesis presents molecular phylogenetic studies of the genus *Sauropus* Blume (Phyllanthaceae) and related genera. In this introduction, the general characters of *Sauropus* and related genera and the economic importance of *Sauropus* are briefly reviewed. The changes in classification of the studied genera and the problems regarding the circumscription of *Sauropus* are discussed. The research questions are presented and the outline of the thesis is provided.

General characters and economic importance of Sauropus species

The most recent revision of *Sauropus* is the study of the Malesian and Thai species (Van Welzen, 2003), which includes species from as well the Australian species radiation as the Southeast Asian centre of speciation Most species of *Sauropus* are herbs to small shrubs (Fig. 1.1a, b). The species are found in waste areas to primary forests and they mainly occur in the lowlands. The leaves of *Sauropus* are simple with entire margins (Fig. 1.1a, b). The stipules are usually small and caducous. The inflorescences of most species are axillary fascicles of one to several flowers (Fig. 1.1b-f). The inflorescensce are very short (Fig. 1.1b, f) to very long (Fig. 1.1c) in several species. The unisexual flowers are usually minute. The calyx of both sexes has six imbricate lobes (Fig. 1.1d, e). The calyx lobes vary between free to completely connate in staminate flowers (Fig. 1.1e, f). Typical are the stamens in the staminate flowers (Fig. 1.1d), which are united and split into three horizontal arms with underneath each arm an anther. The stamens resemble the foot print of a dinosaur, which inspired Blume (1825) to select this name for the genus (Sauros = dinosaur, podus = foot). Also typical are the lack of petals and a disc. The latter may be present in the form of sepal scales in the staminate flowers. The ovaries are generally flat above with three horizontal stigmas on top, which apically split and curve (Fig. 1.1e). The fruits are dehiscent capsules (Fig. 1.1f). 1

Fig. 1.1. Typical characters of *Sauropus* Blume. Habitat of woody herbs growing in fertile area (a) and (b) limestone. A long inflorescence (c) with several staminate and pistillate flowers. Staminate flowers (d) with completely connate calyx lobes and stamens united and split into three horizontal arms. Pistillate flower (e) with three horizontal stigmas on top of the flat ovary, stigmas apically split and curved. Capsular fruit and staminate flowers with free calyx lobes (f). (a: *S. spatulifolius* Beille; b: *Sauropus* "*lithophila*" sp. nov; c: *S. suberosus* Airy Shaw; d & e: *S. discocalyx* Welzen; f: *S. thorelii* Beille).

Several *Sauropus* species have economic significance. Some examples of commercially interesting species are presented here. *Sauropus androgynus* (L.) Merr. is the most important species. Its leaves and young branches are eaten as vegetables and are popular throughout Southeast Asia (Azis, 2003; Hoang et al, 2008). The roots provide medicine (Azis, 2003; Ogle et al., 2003). It is one of the species recommended to eat, because it improves the variety in the vegetable diet and because *S. androgynus* constitutes a high protein source, which is important for those countries where the population has a shortage of animal proteins (Banga, 1956). It also has nutritious value and contains vitamin C and phenolics, which can act as antioxidants (Benjapak et al., 2008). *Sauropus brevipes* Müll.Arg. is used to treat diarrhoea in a decoction with other plants in Peninsular Malaysia (Azis, 2003). *Sauropus macranthus* Hassk. is one of the ornamental plants in Java, its fruits are edible and the leaves are sometimes used as vegetables, just like those of *S. rhamnoides* Blume (Van Welzen, 2003). *Sauropus spatulifolius* Beille is cultivated for its fragrant flowers, just like *S. thorelii* Beille

(Airy Shaw, 1979) and a broth of the leaves and branches of *S. spatulifolius* is drunk to cure a sore throat and cough. The leaves of *Sauropus spatulifolius* are also edible, but have a bitter taste (Van Welzen, 2003). The trunks of *Sauropus villosus* (Blanco) Merr. are used as construction material in the Philippines (Van Welzen, 2003).

Original classification of Sauropus and related genera

Originally, the genus *Sauropus* was classified in the family Euphorbiaceae sensu lato (s.l.), subfamily Phyllanthoideae tribe Phyllantheae subtribe Flueggeinae. In the same subtribe the genera *Breynia* J.R.Forst. & G.Forst., *Glochidion* J.R.Forst. & G.Forst., *Flueggea* Willd., *Margaritaria* L.f., *Phyllanthus* L., *Reverchonia* A.Gray., *Richeriella* Pax & K.Hoffm. (now *Flueggea*), and *Synostemon* F.Muell. can also be found (Webster, 1975). Subtribe *Flueggeinae* contains three genera of which the relationships are not clear: *Breynia*, *Sauropus* and *Synostemon*. *Breynia* is the oldest name (Forster & Forster, 1775), followed by *Sauropus* (Blume, 1826) and *Synostemon* is the youngest name (Mueller, 1858). In 1980, Airy Shaw subsumed the Australian genus *Synostemon* under *Sauropus* and he stated that the closely related *Breynia* is scarcely distinct from *Sauropus* (Airy Shaw, 1980a, b, 1981). The name of the monotypic genus *Breyniopsis* described from Indochina by Beille (1925) already demonstates the difficulties in distinguishing *Sauropu*s and *Breyni*a. Beille (1925) considered the new genus to be closely related to *Breynia*. However, Croizat (1940) convincingly argued that *Breyniopsis* is part of *Sauropus* and the genus was transferred to it, a conclusion that is still valid (Webster, 1994).

Classification changes based on phylogenetic analysis

Molecular phylogenetic studies play an important role in plant systematics nowadays. They resulted in the splitting of the family Euphorbiaceae into five families. The uniovulate subfamilies are considered to constitute the core of the Euphorbiaceae (Euphorbiaceae sensu stricto) except for the tribe Gaearieae, which now forms the Pandaceae. The former two bi-ovulate subfamilies (Oldfieldioideae and Phyllanthoideae) now form the Picrodendraceae and Phyllanthaceae, respectively. The tribe Drypetae within the Phyllanthoideae also received family status, the Putranjivaceae (Judd et al, 1999; APG II, 2003; Wurdack et al., 2004, 2009).

The family Phyllanthaceae is the second largest segregated family, and contains c. 2000 species in 59 presently accepted genera, 10 tribes and two subfamilies (Kathriarachchi et al., 2005; Samuel et al., 2005; Hoffmann et al., 2006). Within the new Phyllanthacae, tribe Phyllantheae in subfamily Phyllanthoideae is the largest tribe. The phylogenetic relationships within this tribe were examined by Kathriarachchi et al. (2006) with a focus on the large genus *Phyllanthus*. The results of nuclear ribosomal ITS and plastid *matK* DNA sequence data confirmed the paraphyly of *Phyllanthus* in its traditional circumscription. Embedded within *Phyllanthus* are the genera *Breynia*, *Glochidion*, *Reverchonia*, and *Sauropus* (including *Synostemon*). To make *Phyllanthus* monophyletic all these genera should be united, which would result in a gigantic and not recognisable *Phyllanthus* with at least 1200 species (Kathriarachchi et al., 2006; Hoffmann et al., 2006). An alternative approach would be to split the gigantic *Phyllanthus* in recognizable, monophyletic subclades with generic status.

Problematic circumscription of Sauropus sensu lato

A preliminary phylogenetic study based on the morphological and pollen data by Van Welzen in 2003 showed problems with the delimitation of *Sauropus* in a broad sense. He found *Breynia*, *Sauropus* s.s. and former *Synostemon* to form a trichotomy (Fig. 1.2), whereby *Sauropus* was not monophyletic. Within *Sauropus* s.s., one former species of *Synostemon*, *Sauropus bacciformis* (L.) Airy Shaw was embedded in it and a part of *Phyllantus*, the species with diploporate colpi, was sister to it. Furthermore, the sections recognized within *Sauropus* s.s. (Pax & Hoffman, 1922; Airy Shaw, 1969; see details in Chapter 4) mainly show a polytomy excepted section *Hemisauropus* which constituted a distinct group with the rest of *Sauropus* s.s.

Later, Kathriarachchi et al. (2006) studied the molecular phylogeny in tribe Phyllantheae (Phyllanthaceae), the results (Fig. 1.3) showed that the species of *Phyllanthus* with diploporate colpi are not grouped together with *Sauropus* s.s. Instead, the study showed the inclusion of *Breynia* within *Sauropus* s.s. with the only representative for former *Synostemon*, *Sauropus elachophyllus* (F.Muell. ex Benth.) Airy Shaw as sister group. However the problematic species, *S. bacciformis*, was not represented in this study. Furthermore, nor were there representatives for all sections in *Sauropus* s.s. as recognized by Airy Shaw (1969).

The most important tools presently available to investigate the evolution of *Sauropus* s.l. and its allies are 'phylogenetic hypothesis' based on DNA sequence data. Phylogenetic trees allow us to visualize in a clear fashion the differences as well as the similarities between groups of organisms or any defined taxonomic unit (Felsenstein, 2004).

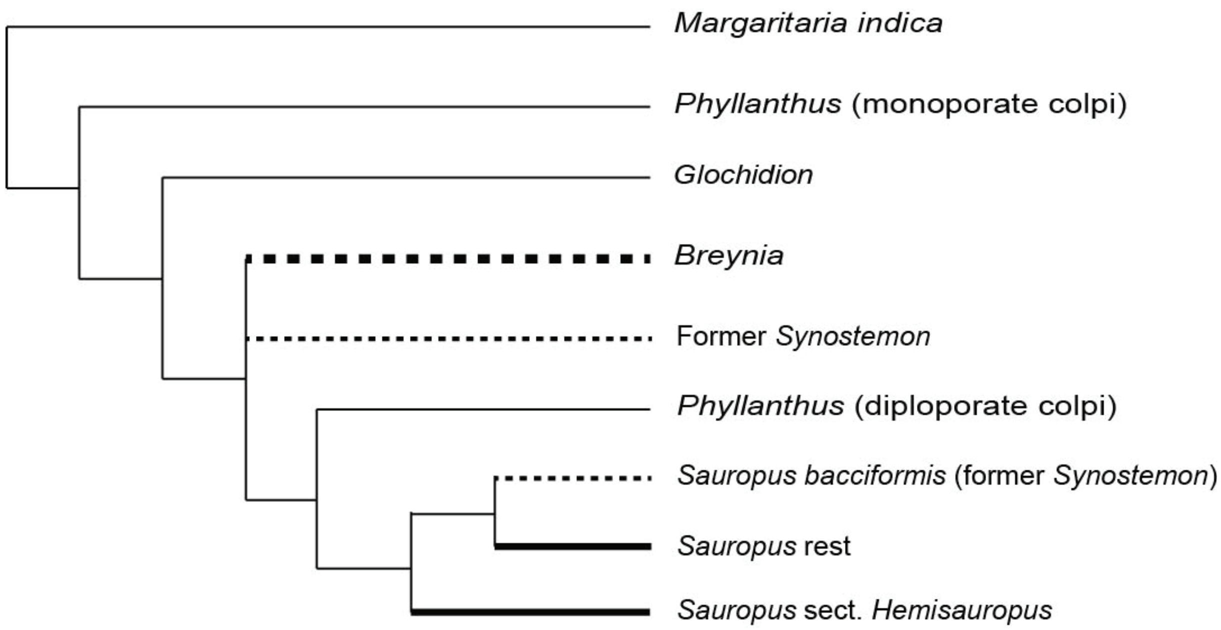

Fig. 1.2. Morphological and pollen phylogeny modified from Van Welzen (2003). Thick line indicates *Sauropus* s.s., thin dashed line indicates former *Synostemon*, thick dashed line indicates *Breynia*, and thin line indicates related genera and outgroup.

Research questions

Therefore, the following main research questions are addressed in this thesis:

1) Are Southeast Asian *Sauropus* s.s. and Australian former *Synostemon* monophyletic?
2) Does former *Synostemon*, *Sauropus bacciformis* group within *Sauropus* s.s. ?
3) Does the molecular phylogeny corroborate the infrageneric groups within *Sauropus* s.s.?
4) What is the phylogenetic position of *Breynia* and *Sauropus*?
5) Can the clades present in the phylogenies be classified as genera and infrageneric taxa, and are they recognisable morphologically?
6) How did *Sauropus* and allied genera evolve geographically?

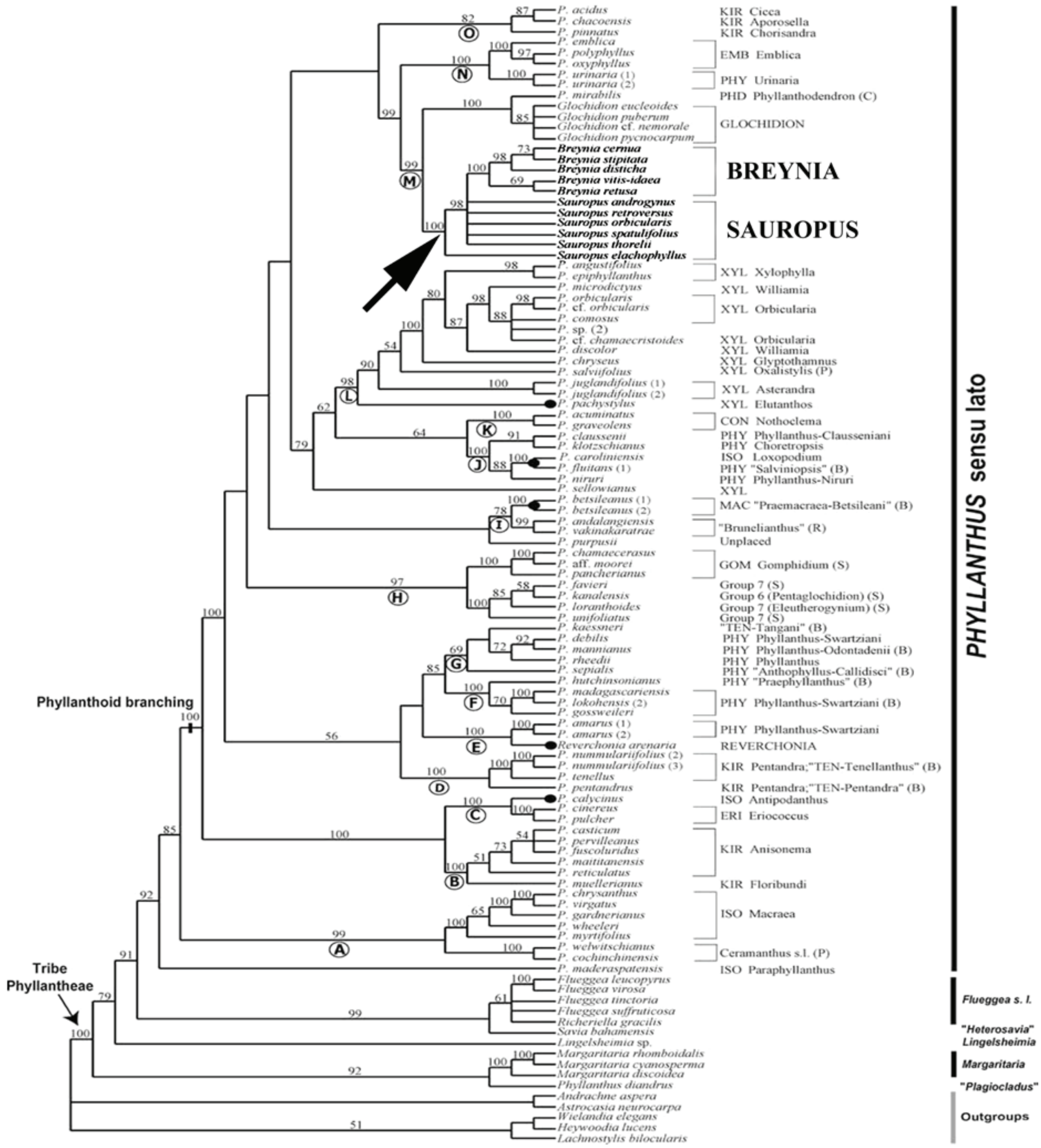

Fig. 1.3. The phylogenetic relationships within the tribe Phyllantheae (Phyllanthaceae) inferred from nr ITS and plastid *matK* (modified from Kathriarachchi et al., 2006). Bootstrap values indicated above branches. Black arrow indicated the position of *Breynia* and *Sauropus*.

Thesis goal and outline

The goal of this Ph.D. research is to focus on the systematics of *Sauropus* and the putatively related genera *Breynia* and *Synostemon*, as well as to resolve their phylogenetic relationships with other related genera.

In chapter 2, the phylogeny of *Sauropus* and related genera is studied using sequence data from plastid *matK* and nuclear ribosomal ITS DNA markers. This chapter pays special attention to the problems concerning the separation of the Australian (former) *Synostemon* and Southeast Asian *Sauropus*.

In chapter 3, the non-coding chloroplast *accD-psaI* and *trnS-trnG* in combination with nuclear ITS and *PHYC* are analysed phylogenetically to further resolve the phylogeny. The results are used to resolve the status of *Sauropus* s.s., former *Synostemon*, and *Breynia* and to delimit infrageneric groups in comparison with the traditional classification within *Sauropus* s.s.

In chapter 4, the molecular data are combined with morphological characters to increase the resolution of the phylogeny and to characterize the various clades. The chapter also makes the nomenclatural changes to transfer all *Sauropus* species to *Breynia*. A new infrageneric classification of this *Breynia* s.l. is presented.

In chapter 5, the results from Chapter 4 are used to reconstruct the historical biogeography of *Sauropus* s.s. and its allies with the aid of S-DIVA.

Chapter 6 concludes and summarises the thesis.